Influence of Seed Head–Attacking Biological Control Agents on Spotted Knapweed Reproductive Potential in Western Montana Over a 30-Year Period

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ABSTRACT Five insect biological control agents that attack flower heads of spotted knapweed, *Centaurea stoebe* L. subsp. *micranthos* (Gugler) Hayek, became established in western Montana between 1973 and 1992. In a controlled field experiment in 2006, seed-head insects reduced spotted knapweed seed production per seed head by 84.4%. The seed production at two sites in western Montana where these biological control agents were well established was 91.6–93.8% lower in 2004–2005 than 1974–1975, whereas the number of seed heads per square meter was 70.7% lower, and the reproductive potential (seeds/m^2) was 95.9–99.0% lower. The average seed bank in 2005 at four sites containing robust spotted knapweed populations was 281 seeds/m^2 compared with 19 seeds/m^2 at four sites where knapweed density has declined. Seed bank densities were much higher at sites in central Montana (4,218 seeds/m^2), where the insects have been established for a shorter period. *Urophora affinis* Frauenfeld was the most abundant species at eight study sites, infesting 66.7% of the seed heads, followed by a 47.3% infestation by *Larinus minutus* Gyllenhal and *L. obtusus* Gyllenhal. From 1974 to 1985, *Urophora* spp. apparently reduced the number of seeds per seed head by 34.5–46.9%; the addition of *Larinus* spp. further reduced seed numbers 84.2–90.5% by 2005. Path analysis indicated that both *Larinus* spp. and *U. affinis* contributed significantly to reduction of seed production over the 30-yr period. Spotted knapweed density may not decrease significantly until the seed bank falls below a critical threshold.

KEY WORDS *Centaurea stoebe*, seed production, seed bank, *Urophora affinis*, *Larinus* spp.

Spotted knapweed, *Centaurea stoebe* L. subsp. *micranthos* (Gugler) Hayek (often reported as *C. maculosa* Lamarck) (Ochsmann 2001), is a perennial plant from Eurasia that has become a serious weed on rangelands of the northwestern United States. First reported in North America in 1893, the plant now infests >3 million ha of rangeland and pasture in 14 states and two Canadian provinces (Lacey 1989, Sheley et al. 1998). The weed infests 1.6 million ha in Montana alone. Spotted knapweed reduces livestock and wildlife forage (Watson and Renney 1974, Thompson 1996), increases surface water runoff and soil sedimentation (Lacey et al. 1989), and reduces plant diversity (Tyser and Key 1988).

Spotted knapweed has been the focus of considerable biological control efforts in Montana. A total of 12 Eurasian insect species have been introduced into Montana for biological control of the plant (Story et al. 2004). Of these, seven species are abundant enough to have an impact on spotted knapweed. Two seed-head flies, *Urophora affinis* Frauenfeld and *U. quadrifasciata* (Meigen) (Diptera: Tephritidae) (introduced in 1973 and 1980, respectively), a seed-head moth, *Metzneria paucipunctella* Zeller (Lepidoptera: Gelechiidae) (introduced in 1980), and two seed-head weevils, *Larinus minutus* Gyllenhal and *L. obtusus* Gyllenhal (Coleoptera: Curculionidae) (introduced in 1991 and 1992, respectively), are well established in many areas of Montana and have a significant impact on spotted knapweed seed production (Story et al. 1989, 1991, Story and Piper 2001, Smith and Mayer 2005). A root moth, *Agapeta zoegana* L. (Lepidoptera: Tortricidae) (introduced in 1984), reduces spotted knapweed biomass (Story et al. 2000), and a root weevil, *Cyphocleonus achates* (Fahraeus) (Coleoptera: Curculionidae) (introduced in 1988), reduces spotted knapweed biomass (Jacobs et al. 2006) and causes mortality to mature spotted knapweed plants (Corn et al. 2006, Story et al. 2006).

Spotted knapweed seed production in Montana has been greatly reduced for many years because of the seed-head insects (Story et al. 1989, 1991, Story and Piper 2001), but the decline in seed production has apparently not affected knapweed density because of the perennial habit of spotted knapweed, its competitive superiority over other plant species, and its large seed bank. Because spotted knapweed populations

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have remained at high densities throughout much of Montana despite the long-term presence of *Urophora* spp., those insects have been considered to be poor agents by some workers because they are abundant but ineffective, thereby increasing their capacity to indirectly impact nontarget species (Ortega et al. 2004, Pearson and Callaway 2006).

Recent observations suggest spotted knapweed density is in decline in many areas of western Montana caused, apparently, by a combination of *C. achates* and other factors (Story et al. 2006). However, the extent to which the long-term impact of the seed-head insects on spotted knapweed seed production has contributed to the decline of the plant in some areas of western Montana has not been measured. We hypothesize that the seed-head insects have reduced seed production for many years, resulting in a gradual reduction of the seed bank that contributed to the reduction of the knapweed population. Specifically, this hypothesis predicts that (1) current spotted knapweed seed production in western Montana should be significantly less than it was in 1974 and 1975, before the widespread establishment of seed-head insects, (2) seed production should be negatively correlated with seed-head insect numbers, and (3) the spotted knapweed seed banks at sites with robust populations should be significantly greater than at sites where the plant has declined.

Materials and Methods

Historical Study. Studies of spotted knapweed seed production were conducted at two sites in the Bitterroot Valley of western Montana during 2004 and 2005. One of the sites (Corvallis) was a 0.2-ha knapweed infestation located 0.8 km northwest of Corvallis, MT, whereas the other site (Missoula) was an 8-ha infestation located at the north edge of Missoula, MT. Spotted knapweed seed heads were collected at both sites in early August, ≈1 wk after flowering, but before seed dispersal. The seed heads were collected along five randomly placed transects at each site. Five seed heads were collected from the nearest knapweed plant at each of 10 points located at 3-m intervals along each transect (=50 seed heads per transect and 250 seed heads per site). The seed heads were dissected in the laboratory and assessed for seed numbers per seed head.

The seed production data at these two sites in 2004 and 2005 were compared with data collected at similar sites in 1974 and 1975 (at the time of the first release of the seed-head insects) and in 1984 and 1985. In 1974, seed production data were collected from four sites near the Corvallis site and one site near the Missoula site. In 1975, data were collected from three of the four 1974 Corvallis area sites and at the same Missoula site. In 1984 and 1985, data were collected from the same four Corvallis area sites used in 1974, one additional Corvallis area site, and at the same Missoula site. The seed production data at the multiple Corvallis area sites in 1974, 1975, 1984, and 1985 were averaged to produce one mean for each year. By 2004, the knapweed at all of the 1975–1985 study sites had been eliminated because of land development, so a new Corvallis site was selected that was as close as possible (within 6.4 km) to the original locations of the 1974–1975 Corvallis area sites. The 2004–2005 Missoula site was within 0.4 km of the Missoula site sampled in 1974–1975 and 1984–1985. Seed-head collection and dissection procedures in 1975 were similar to those described above. The number of spotted knapweed seed heads per square meter at the two sites was determined by measurements from 10 random 50 by 50-cm plots per site in 2005. Annual precipitation at the two sites over the 30-yr period is shown in Fig. 1 (NOAA 2007).

The same seed heads collected at the two sites in August 2004 and 2005 were also examined to note the density of five seed-head insect species: *U. affinis, U. quadrifasciata, M. paucipunctella, L. obtusus,* and *L. minutus.* Because it was very difficult to distinguish between the two *Larinus* species, *Larinus* individuals are hereafter referred to as *Larinus* spp. Data on the seed-head insect numbers in 2004 and 2005 were compared with similar data collected in 1984 and 1985 at the sites described above and in 1977 at one of the 1974–1975 Corvallis area sites and at Missoula.

Mowing Experiment. Attempts to use cages and insecticides for insect exclusion when assessing impact of insects on spotted knapweed seed production have been unsuccessful in the past because of the exclusion of pollinators, inability to use insecticides, and failure to effectively exclude the insects (Story et al. 1989, Smith and Mayer 2005). Preliminary studies have shown that mowing spotted knapweed plants during the summer results in the regrowth of new flower buds, which, because they develop after oviposition by the seed-head insects is completed, produce a normal complement of seeds (unpublished data). We conducted this mowing study in 2006 to document the impact of seed-head insects on spotted knapweed seed production. Spotted knapweed plants growing in five plots at the Western Agricultural Research Center near Corvallis were mowed on 13 June. Each plot consisted of a 7-m portion of a row in a 64 by 41-m spotted knapweed garden, with each plot randomly located in a different row. Fifty randomly collected seed heads from plants in the mowed plots were collected on 8 September, just before seed dispersal, and examined for number of seeds. Fifty seed heads were similarly collected from unmowed plants growing in five similar plots in early August and were similarly examined for seed and insect numbers. Any seed heads from the mowed plots that contained a seed-head insect, because of earlier attack by a late-occurring adult insect, were not included in the sample.

Geographic Study. The spotted knapweed seed bank was assessed at eight sites in western Montana in October 2005, 2 mo after seed dispersal. Four of the sites were locations where the spotted knapweed population was greatly reduced after being heavily infested for at least 30 yr (i.e., between 0 and 0.3 knapweed stems/m^2), whereas the other four sites were

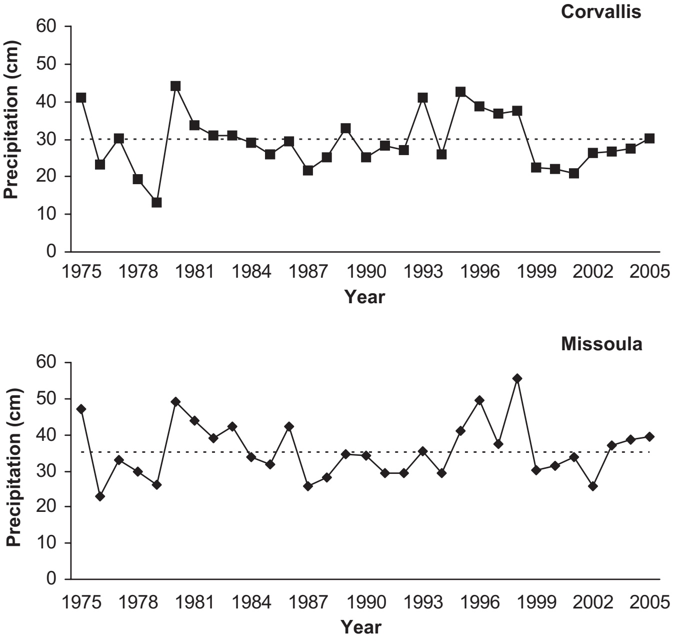

Fig. 1. Annual precipitation at Corvallis and Missoula, MT, from 1975 through 2005. The dashed line represents the long-term average annual precipitation (29.7 cm at Corvallis and 35.1 cm at Missoula).

still infested with spotted knapweed (i.e., between 10.4 and 35.7 knapweed stems/m^2). The four sites with reduced or no spotted knapweed were as follows: Teller Wildlife Refuge (TWR; 2.4 km northwest of Corvallis, MT), Lee Metcalf National Wildlife Refuge (LMNWR; 3 km north of Stevensville, MT), Thomas (3.2 km northeast of Corvallis, MT), and Callan (6.4 km northeast of Corvallis, MT). The four sites with abundant spotted knapweed were as follows: Missoula, Willow Cr. (16 km east of Corvallis, MT), Richardson (16 km northwest of Missoula, MT), and Skalkaho (11 km southeast of Hamilton, MT). In October 2006, the seed bank was also assessed at two spotted knapweed-infested sites in central Montana: one site near Harlowton and the other site near Townsend. Soil cores (6.3 cm diameter by 4.4 cm deep) were collected with a tapered bulb planter along five 25-m, randomly placed transects at each site. At the eight western Montana sites, three soil cores were collected at each of four points located at 5-m intervals along each transect. The three soil cores per interval were combined, resulting in one sample per interval (=4 samples per transect or 20 samples per site). Only one soil core per interval was collected at the two central Montana sites.

The seeds were separated from the soil samples by sifting the soil through a series of sieves with mesh size from 2 to 0.8 mm. Spotted knapweed seed was sorted and identified under a stereo-microscope. Each seed was ruptured using forceps; seeds with moist endosperms were counted as viable seed (DiTomaso et al. 2006).

Spotted knapweed stem density, number of seed heads per square meter, and number of seedlings per square meter at each of the eight western Montana sites were determined by measurements from 15 random 50 by 50-cm plots per site in 2005. Data on the number of seed heads per square meter and seeds per seed head were not collected at the central Montana sites.

Seed heads were collected at each of the four sites with spotted knapweed in western Montana and at the two sites in central Montana to assess seed-head insect numbers and infestation percentage, using the collection procedures described above.

Statistical Analysis. Spotted knapweed seed numbers and insect numbers per seed head were analyzed by analysis of variance (ANOVA) procedures, and means were compared using least significant difference (LSD; Statistix 8 2003). Analysis over time (yr) was done by assigning each sample year to one of the three periods spanned in the study (1974–1977, 1984–1985, 2004–2005). Linear regression analysis was used to describe the relationship between year and spotted knapweed seed production and year and total insect numbers at Corvallis and Missoula. Linear regression was also used to compare the relationship between the various seed-head insect species and to describe the relationship between insect densities and seed bank numbers at the eight sites. Multiple regression analysis was used to describe the effect of individual insect species on seed numbers in the mowing experiment. A logistic curve was used to fit spotted knapweed stems per square meter and seed heads per square meter to seed bank numbers using nonlinear estimation by the Quasi Newton method (StatSoft 1998). Path analysis was conducted following methods in Sokal and Rohlf (1981).

Table 1. Historical changes of reproductive potential of spotted knapweed

Site and year	Mean no. seeds/head ± SEM	Mean no. seed heads/m² ± SEM	Reproductive potential (seeds/m²)
Corvallis 2005	1.4 ± 0.1	261.1 ± 47.8	365.5
Corvallis 2004	3.9 ± 0.2	176.3 ± 39.2	687.6
Corvallis area 1985	14.4 ± 0.9	—	—
Corvallis area 1984	19.2 ± 1.8	—	—
Corvallis area 1975	30.4 ± 1.1	418.8 ± 138.1	12,731.5
Corvallis area 1974	32.9 ± 1.4	—	—
Missoula 2005	2.0 ± 0.2	71.5 ± 26.8	143.0
Missoula 2004	0.8 ± 0.1	146.1 ± 43.2	116.9
Missoula 1985	12.9 ± 1.7	—	—
Missoula 1984	16.5 ± 0.7	—	—
Missoula 1975	19.6 ± 0.9	697.7	13,674.9
Missoula 1974	25.3	—	—

SEM not available for seed head per square meter data in 1975.

Results

Historical Study. Seed numbers per seed head at Corvallis and Missoula were significantly lower with each succeeding decade (F = 91.45; df = 2,6; P = 0.0001; Table 1; Fig. 2). At Corvallis, seed numbers decreased 46.9% from 31.6 seeds per seed head in 1974–1975 to 16.8 in 1984–1985 and 84.2% from 1984 to 1985 to 2.6 seeds per seed head in 2004–2005, with a total decrease of 91.6% over 30 yr. At Missoula, seed numbers decreased 34.5% from 22.45 seeds per seed head in 1974–1975 to 14.7 in 1984–1985 and 90.5% from 1984 to 1985 to 1.4 seeds per seed head in 2004–2005, with a total decrease of 93.8% over 30 yr. Seed numbers per seed head were higher at Corvallis (17.0 ± 5.4 [SE]) than at Missoula (12.8 ± 4.0) over the 30-yr period (F = 7.64; df = 1,6; P = 0.03), but the interaction between site and year was not significant (P = 0.14). The number of seed heads per square meter decreased 70.7% from 558 in 1974–1975 to 164 in 2004–2005 at the two sites (F = 65.07; df = 1,2; P = 0.015), but there was no difference between the sites, nor was the year and site interaction significant. The number of seeds per square meter, which represents reproductive potential, decreased 95.9% from 12,732 in

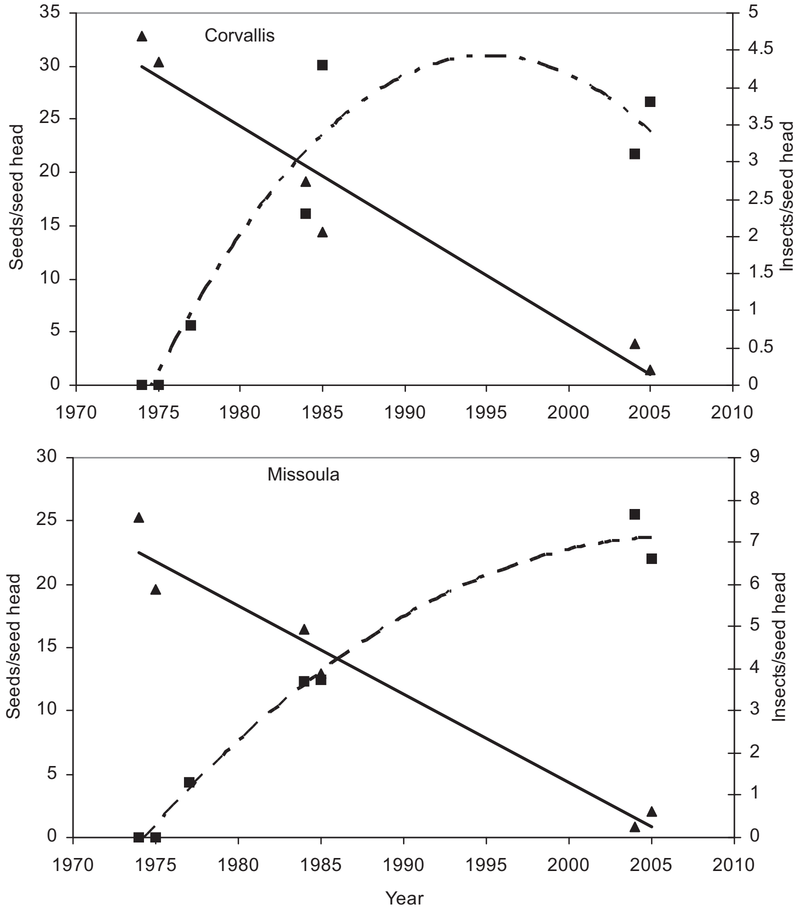

Fig. 2. Association between spotted knapweed seed production decrease and the increase in total seed-head insect numbers at Corvallis and Missoula. The solid line and triangles represent seed numbers, whereas the dashed line and squares represents the insect numbers. Regression equations for Corvallis: for seeds, $y = 1{,}872.5 - 0.9x$, $r^2 = 0.95$, $P < 0.001$; for insects, $y = -42{,}165.3 + 42.3x - 0.01x^2$, $r^2 = 0.89$, $P = 0.03$; regression equations for Missoula: for seeds, $y = 1{,}403.6 - 0.7x$, $r^2 = 0.96$, $P < 0.001$; for insects, $y = -27{,}519.8 + 27.4x - 0.01x^2$, $r^2 = 0.99$, $P = 0.0002$.

Table 2. Historical changes of insect numbers per seed head and percent infestation (mean ± SEM)

Site and year	*Urophora affinis*	*Urophora quadrifasciata*	*Larinus* spp.	*Metzneria paucipunctella*	Total insects
Corvallis 2005	2.5 ± 0.2 (81.7 ± 1.5)[a]	0.6 ± 0.1 (33.6 ± 2.3)	0.7 ± 0.02 (64.7 ± 1.9)	0.007 ± 0.005 (0.7 ± 0.5)	3.8 ± 0.5 (95.9 ± 0.8)
Corvallis 2004	2.0 ± 0.2 (74.4 ± 1.9)	0.4 ± 0.04 (22.7 ± 1.9)	0.7 ± 0.03 (65.4 ± 2.7	0.009 ± 0.002 (0.8 ± 0.1)	3.1 ± 0.4 (95.4 ± 0.7)
Corvallis area 1985	3.7 ± 0.9	0.6 ± 0.2	—	—	4.3 ± 1.5
Corvallis area 1984	2.0 ± 0.8	0.3 ± 0.1	—	—	2.3 ± 0.6
Corvallis area 1977	0.8[b]	—	—	—	0.8
Missoula 2005	5.2 ± 0.2 (92.1 ± 0.6)	0.7 ± 0.04 (30.0 ± 2.1)	0.6 ± 0.04 (52.2 ± 3.4)	0.1 ± 0.02 (12.4 ± 2.0)	6.6 ± 1.2 (98.6 ± 0.4)
Missoula 2004	6.9 ± 0.5 (94.9 ± 0.8)	0.2 ± 0.05 (9.9 ± 2.1)	0.5 ± 0.04 (45.9 ± 3.7)	0.06 ± 0.01 (6.3 ± 1.4)	7.7 ± 1.7 (98.4 ± 0.3)
Missoula 1985	3.7	0.05	—	—	3.7 ± 1.8
Missoula 1984	3.5 ± 0.4	0.2 ± 0.1	—	—	3.7 ± 1.6
Missoula 1977	1.3	—	—	—	1.3

[a] Numbers in parentheses indicate percent infested seed heads.
[b] SEM not available for 1985 at Missoula and 1977 for both sites.

1974–1975 to 527 in 2004–2005 at Corvallis and 99.0% at Missoula (from 13,675 to 130; $F = 8{,}466$; df = 1,2; $P = 0.0001$); site was not significant, but there was a site by year interaction ($F = 22.9$; df = 1,2; $P = 0.041$).

Five species of seed-head insects were present in spotted knapweed seed heads at the Corvallis and Missoula sites in 2004 and 2005 (Table 2). The mean number of total insects per seed head increased successively from 1.05 in 1977 to 3.5 in 1984–1985 to 5.3 in 2004–2005 at the two sites ($F = 17.15$; df = 2,4; $P = 0.01$). Neither site ($P = 0.053$) nor site by year interaction were significant ($P = 0.09$). In 2004 and 2005, the proportion of seed heads infested ranged from 95% at Corvallis to 98% at Missoula.

Urophora affinis was the most abundant insect in terms of numbers per seed head and percentage of infested seed heads. The mean number of *U. affinis* per seed head increased from 0.8 in 1977 to 2.8 in 1984–1985 and 2.25 in 2004–2005 at Corvallis and increased from 1.3 in 1977 to 3.6 in 1984–1985 and 6.05 in 2004–2005 at Missoula ($F = 8.46$; df = 2,4; $P = 0.04$). *U. affinis* numbers per seed head were significantly lower at Corvallis (2.2 ± 0.5) than at Missoula (4.1 ± 0.9) during the 29-yr period ($F = 8.40$; df = 1,4; $P = 0.04$), but the site by year interaction was not significant ($P = 0.11$). The percentage of seed heads infested by *U. affinis* was ≈78% at Corvallis in 2004 and 2005, compared with ≈93% at Missoula (Table 2).

Urophora quadrifasciata numbers per seed head remained at relatively low levels throughout its 27-yr establishment period and did not differ significantly across decades or sites ($P > 0.05$). *U. quadrifasciata* infested ≈28% of the seed heads at Corvallis in 2004 and 2005 compared with ≈20% at Missoula. *Larinus* spp. and *M. paucipunctella* did not become established at either of these sites until after 1985. However, in 2004 and 2005, the *Larinus* spp. infested ≈65% of the seed heads at Corvallis and ≈49% at Missoula. *M. paucipunctella* numbers per seed head were very small at both sites, but the 6 and 12% infestation rates at Missoula in 2004 and 2005 were substantial.

Path analysis of the influence of insect densities to seeds per seed head indicate that only 2.1% of the variance (coefficient of nondetermination) was not explained by the influence of the insects (Fig. 3). *Larinus* spp. had the greatest influence on seed reduction (coefficient −0.969), followed by *U. affinis* (−0.457) and *U. quadrifasciata* (−0.328). The combined effect of the two *Urophora* species (0.885) was nearly as strong as that of *Larinus* spp. *M. paucipunctata* had little influence (+0.209) because of its low abundance, and it is doubtful that its presence increased seed production, as implied by the positive sign of the path coefficient.

Geographic Study. The spotted knapweed seed bank was significantly higher at the four sites containing robust spotted knapweed populations (mean, 280.6 seeds/m^2) than at the four sites where knapweed density has declined (mean, 19.2 seeds/m^2; $F = 6.04$; df = 1,6; $P = 0.04$; Table 3). The seed bank at the sites with robust spotted knapweed populations in western Montana was significantly lower than at spotted knapweed sites in central Montana (mean, 4,218 seeds/m^2; $F = 7.15$; df = 1,4; $P = 0.05$).

The relationship of the density of both spotted knapweed stems and seed heads to seed bank density at the eight sites in western Montana (Fig. 4) was nonlinear and was fit by the following logistic equation: $y = a/\{1 + [a - b]/b \times \exp(-c[x - d])\}$. Parameter a corresponds to the upper plateau, b primarily affects the curvature, c primarily affects the slope, and d is the seed bank density at which the curve starts to rise from 0. For stems per square meter, parameter estimates were as follows: a = 28.97, b = 0.3250, c =

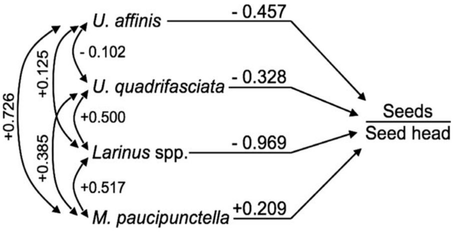

Fig. 3. Path analysis of influence of the abundance of four seed-head insects on the amount of viable seed produced per seed head during the historical study (data from Tables 1 and 2). Values above arrows are path coefficients, indicating the strength of influence. Values beside the double arrows are correlation coefficients used to calculate the path coefficients; ≈98% of the variance in seeds per seed head is explained by the model (unaccounted for variance, $r^2_u = 0.02$).

Table 3. Geographical variation of spotted knapweed seed bank, plant parameters, and insect numbers per seed head (mean ± SEM)

Site	Seed bank (seeds/m²)	Plant parameters (n = 15)			Insect numbers per seed head (n = 250)			
		Stems/m²	Seed heads/m²	Seedlings/m²	*Urophora affinis*	*Urophora quadrifasciata*	*Larinus* spp.	*Metzneria paucipunctella*
Western Montana								
TWR	23.6 ± 9.6	0.3 ± 0.05	3.2 ± 2.1	0	2.5 ± 0.2	0.6 ± 0.1	0.7 ± 0.02	0.007 ± 0.005
Thomas	5.9 ± 5.9	—	—	—	—	—	—	—
LMNWR	47.2 ± 39.7	0.3 ± 0.3	1.1 ± 1.1	4.3 ± 1.8	0.9 ± 0.1	0.06 ± 0.01	0.5 ± 0.04	0.07 ± 0.01
Callan	—	—	—	—	—	—	—	—
Missoula	141.6 ± 34.7	10.4 ± 3.0	71.5 ± 26.8	10.4 ± 2.3	5.2 ± 0.2	0.7 ± 0.04	0.6 ± 0.04	0.1 ± 0.02
Richardson	202.0 ± 32.3	27.2 ± 6.8	144.8 ± 35.3	52.3 ± 8.6	1.6 ± 0.2	0.1 ± 0.04	0.4 ± 0.02	0.1 ± 0.03
Skalkaho	595.9 ± 70.4	24.0 ± 2.8	111.5 ± 20.7	150.4 ± 15.6	1.8 ± 0.2	0.1 ± 0.04	0.4 ± 0.03	0.1 ± 0.02
Willow Cr.	182.9 ± 58.9	35.7 ± 7.0	132.5 ± 16.0	53.6 ± 6.5	1.7 ± 0.2	0.2 ± 0.1	0.4 ± 0.02	0.1 ± 0.04
Central Montana								
Harlowton	758.9 ± 72.8	—	—	—	0.8 ± 0.1	0.1 ± 0.02	0.7 ± 0.05	0
Townsend	7677.8 ± 1630.7	—	—	—	2.4 ± 0.3	0.7 ± 0.2	0.2 ± 0.02	0

0.5002, d = 133.8 (r^2 = 0.95). For seed heads per square meter, parameter estimates were as follows: a = 129.6, b = 0.0772, c = 0.4766, d = 125.6 (r^2 = 0.98). However, seedling density was a linear function of the seed bank density (data not shown; $y = 0.26 \times x - 5.205$; $r^2 = 0.97$; $F = 170.8$; df = 1,6; $P = 0.0001$).

All five species of the seed-head insects were present at the six of eight sites in western Montana that had spotted knapweed seed heads (Tables 3 and 4). *U. affinis* was the most plentiful agent at these sites, infesting 66.7% of the seed heads, followed by the *Larinus* spp. at 47.3%. *U. quadrifasciata* was present in moderate numbers at TWR and Missoula, but occurred in low numbers at the remaining sites, whereas *M. paucipunctella* occurred in low numbers at all sites.

In central Montana, *U. affinis* was well established at Townsend, but occurred in much smaller numbers at Harlowton (Tables 3 and 4). *Larinus* spp. occurred in high numbers at Harlowton, but occurred in low numbers at Townsend. *U. quadrifasciata* occurred at moderate numbers at Townsend, but in low numbers at Harlowton. *M. paucipunctella* was absent at both central Montana sites.

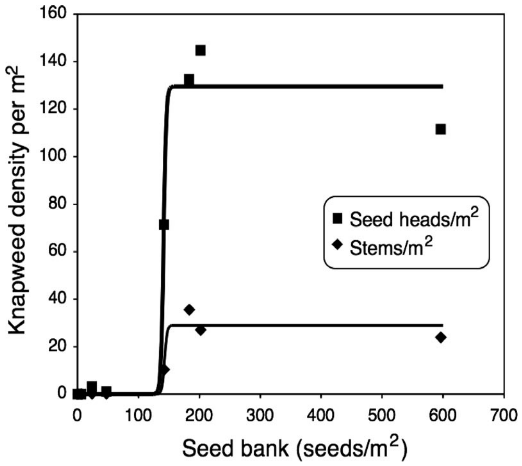

Fig. 4. Relationship of spotted knapweed stem and seed-head densities to seed bank density in the geographic study. The data were fit by the logistic equation (for stems/m², r^2 = 0.95; for seed heads/m², r^2 = 0.98; see text for explanation).

Mowing Experiment. Mean spotted knapweed seed production per seed head in unmowed plots, where insects were abundant, was 84.4% less than in mowed plots, where insects were not present (4.6 ± 0.5 versus 29.4 ± 1.2; $F = 370$; df = 1,8; $P < 0.000$). In unmowed plots, mean number of *U. affinis* per seed head was 1.9 ± 0.3, *Larinus* spp. was 0.9 ± 0.06, *U. quadrifasciata* was 0.2 ± 0.05, and *M. paucipunctella* was 0.1 ± 0.03, for an overall total of 3.1 ± 0.3 insects per seed head. There were no insects in the seed heads from the mowed plots used in this study. Results of multiple regression analysis indicated that each *Larinus* spp. individual reduced knapweed seed production by 12.5 ± 0.6 seeds per seed head compared with 8.3 ± 1.5 seeds for *M. paucipunctella*, 2.8 ± 0.6 seeds for *U. quadrifasciata*, and 1.9 ± 0.2 seeds for *U. affinis* ($Y = 22.9 - 12.5X_1 - 8.3X_2 - 1.9X_3 - 2.8X_4$, where Y = seed production, X_1 = *Larinus* spp., X_2 = *M. paucipunctella*, X_3 = *U. affinis*, and X_4 = *U. quadrifasciata*; $r^2 = 0.59$; $P < 0.001$).

Discussion

The historical study indicated that production of viable seeds per seed head decreased drastically at the two sites during the 30-yr period: 91.6% at Corvallis from 1974–1975 to 2004–2005 and 93.8% at Missoula. The reduction of seeds per seed head seemed to be caused mainly by the high levels of attack by the introduced seed head insects, whose larvae either directly consume seeds or form galls from the ovaries. Path analysis indicated that the *Larinus* species contributed most to reduction of seeds per seed head, but the *Urophora* species were also very important. Seed production decreased from ≈27.0 seeds per seed head at the two sites in 1974–1975 to ≈15.8 in 1984–1985 (41.5% reduction). This reduction was presumably caused by the two *Urophora* species, which were the only abundant seed-head insects present during this period (Table 1). After 1985, the attack rate of *U. affinis* at Missoula increased from ≈3.6 insects per seed head in 1984–1985 to 6.1 in 2004–2005, but it did not increase at Corvallis (2.8–2.2). *U. quadrifasciata* did not increase at either site during this period, and *M. paucipunctella* was not of significance. Therefore,

Table 4. Geographical variation of percentage of spotted knapweed seed heads infested with insects (mean ± SEM)

Site	Percent infestation by insect species (n = 250)				Percent insect-infested seed heads
	Urophora affinis	*Urophora quadrifasciata*	*Larinus* spp.	*Metzneria paucipunctella*	
Western MT					
TWR	81.7 ± 1.5	33.6 ± 2.3	64.7 ± 1.9	0.7 ± 0.5	95.9 ± 0.8
Thomas	—	—	—	—	—
LMNWR	47.3 ± 3.3	4.0 ± 0.8	48.1 ± 4.6	7.3 ± 1.4	76.4 ± 3.1
Callan	—	—	—	—	—
Missoula	92.1 ± 0.6	30.0 ± 2.1	52.2 ± 3.4	12.4 ± 2.0	98.6 ± 0.4
Richardson	54.8 ± 1.9	8.8 ± 1.9	38.4 ± 2.7	14.4 ± 3.2	82.4 ± 2.1
Skalkaho	65.2 ± 4.8	10.4 ± 2.3	42.4 ± 3.2	15.2 ± 2.6	85.6 ± 2.9
Willow Cr.	59.2 ± 4.7	11.5 ± 2.0	38.0 ± 2.3	12.0 ± 4.4	77.2 ± 3.4
Central Montana					
Harlowton	51.6 ± 7.0	8.0 ± 1.9	62.4 ± 4.9	0	88.4 ± 2.7
Townsend	84.4 ± 4.2	31.6 ± 8.6	22.8 ± 2.1	0	92.0 ± 2.1

the further reduction of seed production to ≈2.0 seeds per seed head in 2004–2005 (87.1% reduction from 1984 to 1985) was presumably caused by addition of *Larinus* spp., which became abundant in 2001 (unpublished data).

The results of the mowing experiment indicated that *Larinus* spp. caused a much greater reduction in seed production per individual (12.5 seeds per seed head) than *U. affinis* (1.9) or *U. quadrifasciata* (0.2). However, overall impact depends on the abundance of each of these insect species. The value for the reduction of seeds per insect from the mowing experiment, when multiplied by the number of insects per seed head from the geographic study, provided an estimate of the number of seeds per seed head that were reduced by each species in the latter study. These values were highly correlated to the path coefficients calculated from the historical study (Fig. 5; r^2 = 0.98). Thus, both sets of analyses indicate that *Larinus* spp. contributed the most impact, followed by *U. affinis*, whereas *U. quadrifasciata* and *M. paucipunctella* had little impact.

The density of seed heads per square meter and stems per square meter also decreased over the 30-yr period. Possible causes for these declines could be decreasing water availability, increasing competition with other vegetation, or the impact of insects on vegetative parts of the plant (Story et al. 2001). Water availability, as indicated by annual precipitation (Fig. 1), was higher in 1975 than in 2004–2005, and thus could explain some of the changes in these plant parameters. Adult *Larinus* spp. feed on leaves and stems of knapweeds before they flower and may cause substantial damage at high densities (Jordan 1995, Piper 2004). The root-feeding insects, *A. zoegana* and *C. achates*, first introduced in 1984 and 1988, respectively, probably contributed to the decreases in density of seed heads per square meter and stems per square meter (Story et al. 2000, 2006). Gall formation by *Urophora* spp. can suppress development of other seed heads on the same plant and reduce vegetative growth of diffuse knapweed, *Centaurea diffusa* Lam., which tends to be monocarpic (Harris 1980a, Harris and Shorthouse 1996). Although these effects have not been shown on spotted knapweed, which is polycarpic and longer lived, it is possible that *Urophora* spp. may have contributed partly to the reductions in seed head and stem densities that we observed.

By 2004–2005, the reproductive potential of spotted knapweed (seeds/m^2) had been reduced to 4% of 1974–1975 levels at Corvallis and 1% of 1974–1975 levels at Missoula. Reproductive potential (seeds/m^2) should be a function of seed head density (seed heads/m^2) times production per seed head (seeds/seed head). The reduction in reproductive potential was apparently caused more by a reduction in seeds per

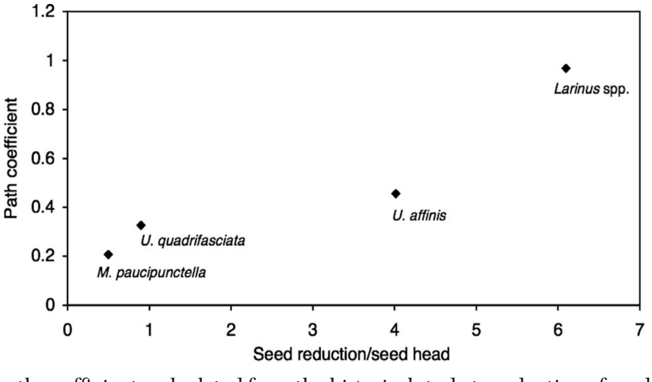

Fig. 5. Correlation of path coefficients calculated from the historical study to reduction of seeds per seed head calculated from the mowing experiment and the geographic study (r^2 = 0.87).

seed head, which decreased by 91.6 and 93.8% at the two sites, than by seed heads per square meter, which decreased by 47.8 and 84.4%. Based on these results, reduction of production per seed head was more important than reduction of seed head production.

Based on the nonlinear relationship between the density of spotted knapweed stems to seed bank density in the geographic study (Fig. 4), a decrease in the density of seeds in the soil seed bank would not result in a corresponding decrease in the abundance of knapweed (represented by the density of stems/m^2 or seed heads/m^2). Instead, the plant density would remain relatively constant until the seed bank reaches a threshold, after which it would quickly drop to a much lower level. The same relationship applies to the seed head density, and both of these variables are correlated to plant biomass (Story et al. 2001). Therefore, when seed-head insect biological control agents reduce production of seeds per square meter and cause a gradual decrease in the density of seeds in the soil, the knapweed population would not be expected to decline until the seed density reaches the threshold. In western Montana, the threshold seems to be $\approx$160 seeds/m^2, although this estimate is not very precise. Regional and temporal differences in rates of survivorship of seeds in the soil, seed germination, and survivorship of young plants likely affect the level of this threshold. Nevertheless, in general, we expect that biological control agents that significantly reduce the availability of seeds would only impact the target plant's population by driving it below a specific threshold. Furthermore, based on the shape of this curve (Fig. 4), we predict that the spotted knapweed population in this region of western Montana would be relatively stable at either high or low densities, corresponding to the two plateaus in Fig. 4. These two plateaus represent two equilibria that correspond to the densities of the plant before and after establishment of effective biological control agents.

There were more seeds in the soil seed banks in central Montana than in western Montana, possibly because seed-head insects have been well established for a shorter period in central Montana (10–15 yr) than in western Montana (>25 yr; Story et al. 1987, Lang et al. 2000). Thus, seed production would have been reduced for a shorter period in central Montana, presumably causing less reduction of the seed bank than occurred in western Montana. If this is the case, perhaps 20 yr of sustained attack by seed-head insects are required to reduce the seed bank sufficiently to achieve acceptable levels of control. However, it is possible that the root-feeding insects, which were released later, could shorten this time by reducing fecundity and survivorship of plants.

Of the five seed-head insects established against spotted knapweed, the seed-head fly, *U. affinis,* was the most abundant agent at all study sites except for Harlowton, where *Larinus* spp. was slightly more abundant. As mentioned earlier, regression data indicate that each *U. affinis* larva reduced seed production by about two seeds per seed head, a number consistent with data collected earlier (Story 1976). However, this number is probably an underestimate of the total impact, because galls of *U. affinis* act as strong metabolic sinks that can reduce seed production in both attacked and unattacked seed heads and may suppress development of other seed heads and reduce vegetative growth (Harris 1980a, Harris and Shorthouse 1996). The fly has been established in sizeable numbers in western Montana for >25 yr, is widespread (Story et al. 1987), and has had a proven impact on seed production (Story et al. 1989).

Interestingly, other workers have suggested that the *Urophora* flies are examples of poor biological control agents because they have established but failed to control their target species, thereby having the potential to become superabundant and to increase their capacity to indirectly affect nontarget species (Ortega et al. 2004, Pearson and Callaway 2005, 2006). *Urophora* spp. have become new food resources for several kinds of vertebrates (Story et al. 1995), potentially affecting food web interactions (Ortega et al. 2004, Pearson and Callaway 2006). In fact, the vertebrate predation has undoubtedly reduced the efficacy of *Urophora* spp. by greatly reducing the number of adults available to attack plants. By reducing *Urophora's* impact on knapweed, such predation has also increased the likelihood of indirect nontarget interactions. However, our data indicate that *U. quadrifasciata* is not abundant and has had limited impact, but *U. affinis,* which is abundant, has been very effective in reducing seed production. Realization of the effectiveness of *U. affinis* and the other biological control agents has been delayed because of the longevity of spotted knapweed plants and the seed. The effect of *Urophora* spp. on food webs is likely to be temporary now that biological control agents are starting to reduce knapweed populations. When the knapweed populations decline, so will the populations of these host-specific insects, thus reducing their contribution to foodwebs (Pearson and Callaway 2005, Smith 2006).

In the mowing experiment, each *Larinus* spp. larva destroyed $\approx$12 seeds per seed head; therefore, although *Larinus* spp. were generally less abundant (insects per seed head sampled) than *U. affinis* at the various field sites, the per insect impact of *Larinus* spp. more than compensated. The highest proportion of seed heads infested by *Larinus* spp. was 65%, which occurred at several sites. When considering that *Larinus* spp. are believed to displace *Urophora* spp. within a seed head (Harris 1990, Smith and Mayer 2005), this insect alone is probably not capable of achieving the high rates of infestation observed by all the insects combined (up to 98%). Therefore, the foraging ability of the two *Urophora* species seems to complement the attack of *Larinus* spp., reducing productivity of seed heads not attacked by the latter insect. *U. quadrifasciata* is considered to be the best disperser (Harris 1980b, Mays et al. 2003) and therefore may play an increasingly important role when spotted knapweed becomes less abundant. The *Larinus* spp., initially introduced in western Montana in 1991 and 1992, have become well established in many areas of western Montana and are undoubtedly playing a major role in the reduction of seed production at this time. The *Larinus* spp. have proven to be effective seed-head agents of diffuse and spotted knapweed in other areas (Smith and Mayer 2005, Crowe and Bourchier 2006). Because of their noticeable impact on seed production, their rapid dispersal, and their ability to escape bird and rodent predation during the winter (because they overwinter in the soil), *Larinus* spp. may become the most important seed-head agents in western Montana once they develop large populations.

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References Cited

Corn, J. G., J. Story, and L. J. White. 2006. Impacts of the biological control agent *Cyphocleonus achates* on spotted knapweed, *Centaurea maculosa,* in experimental plots. Biol. Control 37: 75–81.

Crowe, M. L., and R. S. Bourchier. 2006. Interspecific interactions between the gall-fly *Urophora affinis* Frfld. (Diptera: Tephritidae) and the weevil *Larinus minutus* Gyll. (Coleoptera: Curculionidae), two biological control agents released against spotted knapweed, *Centaurea stoebe* L. ssp. *micranthos.*. Biocontrol Sci. Technol. 16: 417–430.

DiTomaso, J. M., G. B. Kyser, J. R. Miller, S. Garcia, R. F. Smith, G. Nader, J. M. Connor, and S. B. Orloff. 2006. Integrating prescribed burning and clopyralid for the management of yellow starthistle (*Centaurea solstitialis*). Weed Sci. 54: 757–767.

Harris, P. 1980a. Effects of *Urophora affinis* Frfld. and *U. quadrifasciata* (Meig.) (Diptera: Tephritidae) on *Centaurea diffusa* Lam. and *C. maculosa* Lam. (Compositae). Zeitschrift Angewandte Entomol. 90: 190–201.

Harris, P. 1980b. Establishment of *Urophora affinis* Frfld, and *U. quadrifasciata* (Meig.) (Diptera: Tephritidae) in Canada for the biological control of diffuse and spotted knapweed. Zeitschrift Angewandte Entomol. 89: 504–514.

Harris, P. 1990. Feeding strategy, coexistance and impact of insects in spotted knapweed capitula. Proceedings VII. International Symposium on Biological Control of Weeds, 6–11 March 1988, Rome, Italy.

Harris, P., and J. D. Shorthouse. 1996. Effectiveness of gall inducers in weed biological control. Can. Entomol. 128: 1021–1055.

Jacobs, J. S., S. E. Sing, and J. M. Martin. 2006. Influence of herbivory and competition on invasive weed fitness: observed effects of *Cyphocleonus achates* (Coleoptera: Curculionidae) and grass-seeding treatments on spotted knapweed performance. Environ. Entomol. 35: 1590-1596.

Jordan, K. 1995. Host specificity of *Larinus minutus* Gyll. (Col., Curculionidae), an agent introduced for the biological control of diffuse and spotted knapweed in North America. J. Appl. Entomol. 119: 689–693.

Lacey, C. 1989. Knapweed management: a decade of change. Proceedings of the 1989 Knapweed Symposium, 4–5 April 1989, Bozeman, MT.

Lacey, J. R., C. B. Marlow, and J. R. Lane. 1989. Influence of spotted knapweed (*Centaurea maculosa*) on surface runoff and sediment yield. Weed Tech. 3: 627–631.

Lang, R. F., R. D. Richard, P. E. Parker, and L. Wendel. 2000. Release and establishment of diffuse and spotted knapweed biocontrol agents by U.S. Dep. Agric., APHIS, PPQ, in the United States. Pan-Pac. Entomol. 76: 197–218.

Mays, W. T., and L. T. Kok. 2003. Population dynamics and dispersal of two exotic biological control agents of spotted knapweed, *Urophora affinis* and *U. quadrifasciata* (Diptera: Tephritidae), in southwestern Virginia from 1986 to 2000. Biol. Control 27: 43–52.

[NOAA] National Oceanic and Atmospheric Administration. 2007. Annual Climatological Summary: (http://cdo.ncdc.noaa.gov/ancsum/ACS?stnid=20012392 and http://cdo.ncdc.noaa.gov/ancsum/ACS?stnid=20012503).

Ochsmann J. 2001. On the taxonomy of spotted knapweed (*Centaurea stoebe* L.). Proceedings of the First International Knapweed Symposium of the Twenty-First Century, 15–16 March 2001, Coeur d'Alene, ID.

Ortega, Y. K., D. E. Pearson, and K. S. McKelvey. 2004. Effects of biological control agents and exotic plant invasion on deer mouse populations. Ecol. Appl. 14: 241–253.

Pearson, D. E., and R. M. Callaway. 2005. Indirect nontarget effects of host-specific biological control agents: implications for biological control. Biol. Control 35: 288–298.

Pearson, D. E., and R. M. Callaway. 2006. Biological control agents elevate hantaviurs by subsidizing deer mouse populations. Ecol. Lett. 9: 443–450.

Piper, G. L. 2004. Biotic suppression of invasive weeds in Washington state: a half-century of progress. Proceedings of the XI International Symposium on Biological Control of Weeds, 27 April to 3 May 2003, Canberra, Australia.

Sheley, R. L., J. S. Jacobs, and M. F. Carpinelli. 1998. Distribution, biology, and management of diffuse knapweed (*Centaurea diffusa*) and spotted knapweed (*Centaurea maculosa*). Weed Technol. 12: 353–362.

Smith, L. 2006. Cause and effect, and how to make a better biocontrol agent. Biocontrol News Inform. 27: 28N–30N.

Smith, L., and M. Mayer. 2005. Field cage assessment of interference among insects attacking seed heads of spotted and diffuse knapweed. Biocontrol Sci. Technol. 15: 427–442.

Sokal, R. R., and F. J. Rohlf. 1981. Biometry, 2nd ed. W. H. Freeman, San Francisco, CA.

Statistix 8. 2003. Analytical Software, Tallahassee, FL.

StatSoft. 1998. Statistica for Windows, release 5.1. StatSoft, Tulsa, OK.

Story, J. M. 1976. A study of *Urophora affinis* (Diptera: Tephritidae) released on spotted knapweed in western Montana. MS thesis, Montana State University, Bozeman, MT.

Story, J. M., and G. L. Piper. 2001. Status of biological control efforts against spotted and diffuse knapweed. Proceedings of the First International Knapweed Symposium of the Twenty-First Century, 15–16 March 2001, Coeur d'Alene, ID.

Story, J. M., R. M. Nowierski, and K. W. Boggs. 1987. Distribution of *Urophora affinis* and *U. quadrifasciata,* two flies introduced for biological control of spotted knapweed (*Centaurea maculosa*) in Montana. Weed Sci. 35: 145–148.

Story, J. M., K. W. Boggs, and R. M. Nowierski. 1989. Effect of two introduced seed head flies on spotted knapweed. Mont. AgResearch 6: 14–17.

Story, J. M., K. W. Boggs, W. R. Good, P. Harris, and R. M. Nowierski. 1991. *Metzneria paucipunctella* Zeller (Lepidoptera: Gelechiidae), a moth introduced against spotted knapweed: its feeding strategy and impact on two introduced *Urophora* spp. (Diptera: Tephritidae). Can. Entomol. 123: 1001–1007.

Story, J. M., K. W. Boggs, W. R. Good, L. J. White, and R. M. Nowierski. 1995. Cause and extent of predation on *Urophora* spp. larvae (Diptera: Tephritidae) in spotted knapweed capitula. Environ. Entomol. 24: 1467–1472.

Story, J. M., W. R. Good, L. J. White, and L. Smith. 2000. Effects of the interaction of the biocontrol agent, *Agapeta zoegana* L. (Lepidoptera: Cochylidae), and grass competition on spotted knapweed. Biol. Control 17: 182–190.

Story, J. M., L. Smith, and W. R. Good. 2001. Relationship among growth attributes of spotted knapweed (*Centaurea maculosa*) in western Montana. Weed Tech. 15: 750–761.

Story, J. M., G. L. Piper, and E. M. Coombs. 2004. Knapweeds, pp. 196–232. *In* E. M. Coombs, J. K. Clark, G. L. Piper, and A. F. Cofrancesco, Jr. (eds.), Biological control of invasive plants in the United States. Oregon State University Press, Corvallis, OR.

Story, J. M., N. W. Callan, J. G. Corn, and L. J. White. 2006. Decline of spotted knapweed density at two sites in western Montana with large populations of the introduced root weevil, *Cyphocleonus achates* (Fahraeus). Biol. Control 38: 227–232.

Thompson, M. J. 1996. Winter foraging responses of elk to spotted knapweed removal. Northwest Sci. 70: 10–19.

Tyser, R. W., and C. H. Key. 1988. Spotted knapweed in natural area fescue grasslands: an ecological assessment. Northwest Sci. 62: 151–160.

Watson, A. K., and A. J. Renney. 1974. The biology of Canadian weeds. *Centaurea diffusa* and *C. maculosa*.. Can. J. Plant Sci. 54: 687–701.

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